Unconventional Functions for Clathrin, ESCRTs, and Other Endocytic Regulators in the Cytoskeleton, Cell Cycle, Nucleus, and Beyond: Links to Human Disease

Frances M. Brodsky¹, R. Thomas Sosa², Joel A. Ybe³, and Theresa J. O'Halloran²

¹Department of Bioengineering and Therapeutic Sciences, Departments of Pharmaceutical Chemistry and Microbiology and Immunology, The G.W. Hooper Foundation, University of California, San Francisco, San Francisco, California 94143-0552

²Department of Molecular Biosciences, Institute for Cellular and Molecular Biology, University of Texas, Austin, Texas 78712-1095

³Department of Molecular and Cellular Biochemistry, Indiana University, Bloomington, Indiana 47405

Correspondence: t.ohalloran@mail.utexas.edu

The roles of clathrin, its regulators, and the ESCRT (endosomal sorting complex required for transport) proteins are well defined in endocytosis. These proteins can also participate in intracellular pathways that are independent of endocytosis and even independent of the membrane trafficking function of these proteins. These nonendocytic functions involve unconventional biochemical interactions for some endocytic regulators, but can also exploit known interactions for nonendocytic functions. The molecular basis for the involvement of endocytic regulators in unconventional functions that influence the cytoskeleton, cell cycle, signaling, and gene regulation are described here. Through these additional functions, endocytic regulators participate in pathways that affect infection, glucose metabolism, development, and cellular transformation, expanding their significance in human health and disease.

The discovery and characterization of clathrin (Pearse 1975) initiated molecular definition of the many endocytosis regulators described in this collection, which mediate the clathrin-dependent and -independent pathways for membrane internalization (see Kirchhausen et al. 2014; Mayor et al. 2014; Merrifield and Kaksonen 2014). In accompanying reviews, we have seen how these endocytic pathways influence

nutrition and metabolism (see Antonescu et al. 2014), signal transduction (see Bökel and Brand 2014; Di Fiore and von Zastrow 2014), neuronal function (see Morgan et al. 2013; Cosker and Segal 2014), infection and immunity (see ten Broeke et al. 2013; Cossart and Helenius 2014), tissue polarity and development (see Eaton and Martin-Belmonte 2014; Gonzalez-Gaitan and Jülicher 2014), and migration

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and metastasis (see Mellman and Yarden 2013). Recently, it has been established that some endocytic regulators have molecular properties that expand their functions beyond endocytosis. These include molecular interactions that affect the microtubule and actin cytoskeletons, nuclear translocation that influences gene regulation, and the formation of membrane-associated scaffolds that serve as signaling and sorting platforms. Through these diverse nonendocytic functions, endocytosis regulators play additional roles in cell division, pathogen infection, cell adhesion, and oncogenesis. In this article, we review the nonconventional behavior of endocytic regulators, first discussing the molecular properties that enable their moonlighting functions and then discussing the cellular processes and disease states that are influenced by these functions.

NOVEL INTERACTIONS AND BIOCHEMISTRY OF ENDOCYTIC REGULATORS

Clathrin

The polyhedral lattice of the conventional clathrin coat is formed by self-assembly of the three-legged triskelion-shaped molecule made up of three clathrin heavy-chain (CHC17) subunits, encoded by the *CTLC* gene on human chromosome 17 (for a review of clathrin biochemistry, see Brodsky 2012; Kirchhausen et al. 2014). The CHC17 triskelion has bound clathrin light-chain (CLC) subunits that span the central portion from the vertex to the bend in the knee. CLC confers stability to the trimerization domain of the triskelion and rigidity to the proximal leg portion, and its binding to the triskelion knee via a labile salt bridge can straighten the knee to an angle that is incompatible with assembly, enabling negative regulation of clathrin self-assembly (Wilbur et al. 2010b). The CLC subunit also links the actin-organizing huntingtin-interacting protein 1 (Hip1) and its related mammalian protein Hip1R or the yeast homolog Sla2p to the clathrin lattice (Brodsky 2012).

Clathrin with bound CLC is the active form for endocytosis and vesicular transport for all

eukaryotes. When vertebrates emerged, a second isoform of clathrin was enabled by gene duplication, generating the *CTCL1* gene on human chromosome 22 (and homologous genes in other species) encoding the CHC22 heavy chain. This gene is present in most vertebrates but has degenerated into a pseudogene in mice. The encoded CHC22 protein is 85% identical in sequence to CHC17, with differences equally distributed along the length of the protein. CHC22 trimerizes but does not bind CLC. In spite of being missing from mice, CHC22 clathrin plays a key nonendocytic role in humans, mediating retrograde membrane traffic from late endosomes to the *trans*-Golgi network (TGN) (Esk et al. 2010). This pathway generates the intracellular storage compartment for the insulin-responsive GLUT4 glucose transporter in fat and muscle, where CHC22 is expressed at its highest levels (Vassilopoulos et al. 2009), and is a minor pathway in other tissues where CHC22 is present at low levels. Studies to date suggest that CHC22 clathrin is more stable in its membrane association than CHC17 and that its biochemical behavior is different because of its lack of CLC association and more limited interaction with adaptor molecules (Liu et al. 2001).

The amino-terminal domain of CHC17 clathrin binds the AP1, AP2, AP3, and GGA adaptor molecules, but it can also participate in microtubule binding (Royle 2012). At cell cycle onset, Aurora kinase A activation phosphorylates TACC3, a microtubule-stabilizing protein. Phosphorylated TACC3 interacts with the terminal domain of CHC17, and these proteins join chTOG in a complex that stabilizes microtubule-based structures including centrosomes and the kinetochore fibers of the mitotic spindle (Booth et al. 2011; Foraker et al. 2012). Thus, CHC17 decorates the mitotic spindle in mammalian cells, and its presence contributes to centrosome integrity. Although there is some debate about the molecular details of interaction within the CHC17–TACC3–chTOG complex, it is clear that this complex plays a cellular role in microtubule function that is completely distinct from the membrane traffic function of clathrin and that this role

begins in S phase upon Aurora A kinase activation (Cheeseman et al. 2011; Foraker et al. 2012). Kinetochore microtubule stabilization by the CHC17–TACC3–chTOG complex requires CHC17 trimerization, and the complex appears to act as a cross-bridge within microtubule bundles, exploiting the multimeric properties of CHC17 (Booth et al. 2011). The evolutionary extent of CHC17 association with TACC3 and chTOG is not clear, but spindle-associated clathrin is evident in *Drosophila*, as well as all vertebrates that have been examined.

CHC17 clathrin is also capable of nuclear translocation, where it can influence transcriptional activation by p53. For CHC17 to translocate, the conventional clathrin trimeric molecule must be made monomeric. Dissociation of CLC and consequent destabilization of the carboxy-terminal trimerization domain of CHC17 increases levels of CHC17 monomer, although this is still a minor form of CHC17 in the cell. Atomic-level details of what structural elements contribute to the stability of the CHC17 trimer were revealed by the X-ray structure of the isolated trimerization domain (CTXD) without bound CLC (Fig. 1) (PDB code 3QIL) (Ybe et al. 2013). In the structure, the position of Helix 7j is shifted by ~ 10 Å toward Helix 7h compared with lower-resolution models with bound CLC (Fotin et al. 2004; Wilbur et al. 2010b). In the structures with bound CLC (assembled clathrin [PDB code 1X14] [Fotin et al. 2004] and clathrin hub [PDB code 3LVG] [Wilbur et al. 2010b]), the space between Helices 7j and 7h is occupied by CLC density, suggesting that the orientation of Helix 7j is dependent on CLC (Wilbur et al. 2010b; Ybe et al. 2013). The CTXD structure raises new questions of what can happen to the global topology of trimeric clathrin if CLC is not present or if the carboxyl terminus of CLC that contributes to the stability of the trimerization domain (Ybe et al. 2007b) is moved away. It is important to point out that the impact of CLC on clathrin trimer stability varies in different organisms. CLC knockouts in *Saccharomyces cerevisiae* but not in *Dictyostelium* (Wang et al. 2003) result in detramerization (Chu et al. 1996; Huang et al. 1997). The details of how nontrimeric clathrin enters the nucleus

are not yet known, but the binding of monomeric CHC17 to p53 suggests that entry may be facilitated by the nuclear translocation signal in p53. It will also be important to find out whether there is any regulatory role for CLC. The evidence so far suggests that the binding of p53 is only possible when CLC is not bound to CHC17 (Enari et al. 2006). The detramerization switch hypothesis (Fig. 1) offers a novel mechanism to convert the topology of clathrin for new function in the nucleus.

Endocytic Accessory Proteins

Many endocytic accessory proteins, including eps15 and epsin, are known to translocate to the nucleus, although their function there remains poorly understood (Benmerah 2004; Pilecka et al. 2007). Among those with implicated function is Hip1, which in addition to its role in linking CLC to the actin cytoskeleton, can also piggyback on androgen receptor (AR) to get inside the nucleus to regulate transcription (Mills et al. 2005). Hip1 is a member of the HIP family of proteins that includes Hip1R (R for related), the *Dictyostelium* Hip homolog, and yeast Sla2p. Originally identified as a binding partner of huntingtin in the brain (Kalchman et al. 1997; Wanker et al. 1997), Hip1 is a multidomain dumbbell-shaped protein that exists as a parallel homodimer (Engqvist-Goldstein et al. 2001). Hip1 binds to phospholipids through an ANTH (AP 180 amino-terminal homology) domain in its amino terminus (Mishra et al. 2001; Legendre-Guillemin et al. 2004). Hip1 contains FXDXF and DPF motifs (X denotes any amino acid) to bind AP2 and has a clathrin box (₃₃₂LMDMD) that interacts with the amino-terminal domain of clathrin in vitro (Brett et al. 2002). The central coiled-coil of the Hip1 molecule serves as a dimerization domain and is inherently flexible (Ybe et al. 2007b; Niu and Ybe 2008; Wilbur et al. 2010a; Fontaine et al. 2012). The structure of Hip1 (PDB 2NO2) reveals that the CLC binding surface originally mapped to the ₄₈₄DLLRKN region (Legendre-Guillemin et al. 2005) is composed of a short solvent-exposed hydrophobic patch that is interrupted by a basic patch centered on K494

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Figure 1. Topology switch that disrupts clathrin trimerization. The clathrin topology switch is proposed to have a top and a bottom latch that hold the triskelion together. The formation of monomeric and dimeric clathrin requires these latches to be opened. Monomeric clathrin can translocate to the nucleus for nonendocytic clathrin functions. (A) The top view of the clathrin trimerization domain structure (PDB 3QIL) shows that each heavy-chain leg contains its own topology switch comprising Helix 7j (teal), TX1 (green), and pCs (red, predicted to undergo coil-to-strand transition). The interface between neighboring legs (dashed lines) involves pCs in one leg and TX1 in the next leg over. (B) The top latch elements of the topology switch (same color coding as in A). Helix 7j in 3QIL without the light chain is moved by ~ 10 Å compared with the same helix (gray) in a structure with the light chain (PDB 1X14). This suggests that the light chain could be involved in determining the orientation of Helix 7j. The red strand pCs is coiled in 1X14 (also in 3LVG). This is significant because this stretch—predicted to undergo a strand-to-helix transition—would impact the interface between the legs in A if its conformation changes. (C) The bottom latch of the topology switch in the helix tripod of the trimerization domain must be broken to completely free a leg. The distortion in 3QIL is partly due to disruptions in the helix tripod (see arrows) when the helix tripod of 3QIL is compared with that of 1X14. (D) Cartoon of a eukaryotic cell shows a pool of trimeric clathrin separate from that participating in clathrin-mediated endocytosis (CME). This population detrimerizes (step 1) to yield a mixture of monomers and dimers with bound light chain (CLC, red). Dimers may be useful as is or further converted to monomeric clathrin (step 2). Monomers enter the nucleus in step 3. It is also possible that CLC is first released in the cytosol (step 4) before entry in step 5 (yellow). In this hypothesis, step 4 and 5 produce a mixed nuclear pool of monomeric clathrin with and without the light chain. Although CLC-free monomeric clathrin can interact with p53 in the nucleus, further studies are needed to fully understand the function of nuclear clathrin.

(Ybe et al. 2007a). There is a second CLC binding determinant (K474) upstream from the DLLRKN region (Ybe et al. 2009). The carboxy-terminal region of Hip1 (the crystal structure of the Hip1R region is known; PDB 1R0D) (Brett et al. 2006) has a THATCH domain that tethers clathrin-coated vesicles to the cellular cytoskeleton. Immunoprecipitation studies indicate the Hip1 coiled-coil domain can bind androgen receptor (AR), and confocal microscopy shows a significant redistribution of Hip1 to the nucleus, where it regulates AR transcription (Mills et al. 2005).

Hip1 can also influence cellular apoptosis when bound to an accessory protein that is predicted to contain a death effector domain (DED). HIPPI (Hip1 protein interactor) forms a proapoptotic complex with Hip1 that some have suggested contributes to the apoptotic death of specific neurons in Huntington's disease (Hackam et al. 2000; Gervais et al. 2002; Niu and Ybe 2008). The crystal structure of the HIPPI-binding domain of Hip1 (PDB 2QA7) suggests that the formation of the HIP1/HIPPI complex is mediated by the flexibility of Hip1 (Niu and Ybe 2008). There are conflicting data in the literature about the ability of HIP1/HIPPI to activate both intrinsic (caspase-8 independent) and extrinsic (caspase-8 dependent) cell death pathways (Hackam et al. 2000; Gervais et al. 2002). The fact that alternative splicing of the *HIP1* gene yields two splice variants (Chopra et al. 2000) could reconcile why both pro- and antiapoptotic effects involving Hip1 have been reported (Rao et al. 2001).

The Numb protein binds the AP2 endocytic adaptor, but it also translocates to the nucleus, where it has been implicated in growth control and breast cancer metastasis. Best known for its role in regulating the Notch signaling pathway, the Numb protein is portioned asymmetrically into the daughter cells during *Drosophila* neurogenesis. In cells inheriting Numb, Numb directly binds to the Notch receptor and α -adapten of the AP2 complex, serving as a scaffold to promote Notch internalization and degradation (Guo et al. 1996; Santolini et al. 2000). Thus, preferential inheritance of Numb regulates Notch distribution and signaling, thereby suppressing the

proliferative potential of the Notch signaling pathway (Berdnik et al. 2002; Wirtz-Peitz et al. 2008). Numb also undergoes nucleocytoplasmic shuttling by binding to the Mdm2 protein, allowing Numb to have a nuclear function independent of its endocytic regulation of Notch signaling (Scita and Di Fiore 2010).

The endosomal APPL protein (adaptor protein-containing PH domain, PTB domain, and leucine zipper motif) also translocates into the nucleus. APPL1 and APPL2 function in the endocytic pathway as effectors of the early endosomal protein Rab5 (Miaczynska et al. 2004). After GTP-Rab5 hydrolyzes its GTP, APPL dissociates from the Rab5 endosomes and translocates into the nucleus. Although APPL1 does not contain a nuclear localization signal (NLS), its interactions with nuclear proteins through its PH domain may target it to the nucleus. APPL2 contains a PH domain and a putative NLS that could facilitate its nuclear localization (Miaczynska et al. 2004).

In addition to endocytic roles, the clathrin assembly lymphoid myeloid (CALM) protein also has nuclear roles. In endocytosis CALM binds to several components of the clathrin coat, including clathrin, AP2, and PIP₂ (Ahle and Ungewickell 1986; Tebar et al. 1999; Ford et al. 2001; Huang et al. 2004). Here CALM functions to regulate endocytic and endosomal trafficking. However, CALM also plays a role in transcriptional regulation. CALM does not contain a nuclear localization signal, yet has been shown to undergo nuclear translocation (Vecchi et al. 2001). This could be mediated by interactions between CALM and CATS (CALM-interacting protein expressed in thymus and spleen) (Archangelo et al. 2006). Despite lacking a canonical nuclear localization signals, CATS preferentially localizes to the nucleus. Thus, CATS may promote nuclear import of CALM, providing a means for CALM to function as a transcriptional regulator.

ESCRTs

Proteins in the ESCRT (endosomal sorting complex required for transport) complexes conventionally function in degradative pathways by

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sorting ubiquitinated cargo and inducing the formation of multivesicular bodies (MVBs) (see Henne et al. 2013). In brief, this pathway involves the interaction of ESCRT-0 (also known as Hrs) with clathrin and sequestration of ubiquitinated cargo at endosomes, which triggers sequential recruitment of complexes of Vps proteins known as ESCRT-II and -III, leading to membrane deformation and MVB budding. This is followed by recruitment of the Vps4–Vta1 complex, which mediates ESCRT dissociation from membranes (Henne et al. 2011). ESCRTs also act in nonendocytic functions including the budding of enveloped viruses from cells, as well as promoting the final stage of membrane scission during cytokinesis (McDonald and Martin-Serrano 2009; Hurley 2010). What these seemingly disparate cellular pathways have in common is that they all share topologically similar fusion events. In MVB formation, small vesicles bud into the endosome lumen, and in viral budding, the enveloped virus buds from the cytosol into an endosome-like compartment that is connected to the cell surface. In cytokinesis, when a daughter cell separates from its sister, formation of the abscission furrow requires a similar bending of membranes (Fig. 2). Despite topological similarities, the precise mechanisms differ somewhat for each process as the particular ESCRT complex(es) involved vary for these different pathways (Peel et al. 2011; Morita 2012).

PHYSIOLOGICAL AND DISEASE-RELATED CONSEQUENCES

Having described the biochemistry of endocytic regulators that facilitates their nonendocytic functions, we now address the physiological pathways and disease states that are influenced by these functions. We also summarize the novel functions mediated by individual proteins in Table 1.

Organizing Actin for Adhesion, Bacterial Infection, and Immunity

The CLC–Hip protein–actin interaction is important for clathrin-mediated endocytosis from

Figure 2. ESCRT proteins function in similar membrane fission events. (*Left*) The endosomal sorting complexes required for transport (ESCRT) complexes have nonendocytic roles in viral budding (A), multivesicular body (MVB) formation (B), and cytokinesis (C). (*Right*) The outward bending of the plasma membrane enveloped around a virus (A') and vesicles budding into the lumen of multivesicular bodies (B') require membrane fusion leading to fission. Similarly, ESCRTs induce closely apposed membranes (C') to fuse and liberate the midbody during cytokinesis.

membranes under tension such as in polarized cells in culture and presumably most tissues (Boulant et al. 2011). CLC–Sla2p interaction is also required for endocytosis from membranes under turgor pressure such as the yeast plasma membrane and the contractile vacuole membrane in *Dictyostelium* cells (Archangelo et al. 2006; Stavrou and O'Halloran 2006; Aghamohammadzadeh and Ayscough 2009). The interaction of CLC with actin via Hip proteins and the additional binding of Hip1R to cortactin in metazoans confer an additional role on the clathrin lattice as an actin organizer during adhesion and infection by large viruses or bacteria (see Cossart and Helenius 2014). These pathways represent situations in which the clathrin lattice itself is not performing an endocytic function but acting as a platform to recruit the Hip proteins through their interaction with

Unconventional Functions of Endocytosis Regulators

Table 1. Nonendocytic functions of endocytic regulators

Endocytic regulator	Nonendocytic function	Pathway significance
Clathrin-CHC17 isoform (CLC bound)	Microtubule binding in a complex with TACC3 and chTOG on the mitotic spindle and in centrosomes	Cancer/cell cycle
	Nuclear translocation and p53 transcription	Cancer
	Platform for actin organization during viral and bacterial infection and adherens junction formation (dependent on CLC and Hip proteins)	Infection/development
	Cytokinesis (also a role for dynamin)	Cancer/cell cycle
Clathrin-CHC22 isoform (no CLC)	Retrograde sorting at the endosome for cargo targeted to the TGN and to the insulin-responsive GLUT4 compartment	Metabolism/type II diabetes
Hip1	Androgen receptor binding and nuclear translocation for transcriptional regulation	Cancer
	Apoptosis effector via HIPPI binding	Cancer/development
Numb	Nuclear translocation and influence on growth control and p53 transcription	Cancer/development
	Regulation of Notch signaling	Signaling
APPL proteins	Nucleosome remodeling and transcriptional control	Signaling
CALM	Transcriptional regulation	Cancer
ESCRT complexes	Abscission in cytokinesis	Cell cycle
	Virus budding	Infection

CLC in order to influence local actin cytoskeleton arrangements.

During the initial stages of adherens junction (AJ) formation, clathrin is recruited to sites of cell–cell interaction through its interaction with cadherin in the mammalian junction (Bonazzi et al. 2012). If this recruitment is inhibited, actin cables do not properly form at the AJ. Normal actin morphology at the AJ also depends on the presence of the CLC and Hip proteins and is concomitant with tyrosine phosphorylation of CHC17. *Listeria monocytogenes* exploits this pathway for intracellular infection by binding to cadherin through its Internalin A protein. Binding induces CHC17 phosphorylation, which is required for actin recruitment via CLC–Hip protein interactions (Bonazzi et al. 2011). Actin then surrounds the clathrin associated with cell-bound *Listeria* and mediates internalization of the organism, which is too large to enter cells through clathrin-coated vesicles, the latter being limited to

200 nm in diameter. CHC17 clathrin is comparably used during formation of actin pedestals during pathogenic interaction of enteropathogenic *Escherichia coli* (EPEC) with host cells. CHC17 is phosphorylated at the bacterium–host interface and recruits a clathrin-associated layer of actin, which forms a pedestal structure in the host cell enabling transfer of pathogenic molecules from the attached bacterium into the host cytoplasm (Bonazzi et al. 2011).

Tyrosine phosphorylation of CHC17 was first observed in response to epidermal growth factor (EGF) binding to its receptor and was shown to be a function of SRC family kinase signaling downstream from receptor tyrosine kinase activation (Wilde et al. 1999). SRC family kinase activation by the T-cell receptor and B-cell receptor (surface immunoglobulin) on lymphocytes also induces CHC17 phosphorylation and is required for endocytic clearance of these receptors (Stoddart et al. 2002; Crotzer et al. 2004). The target phosphorylation sites are

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tyrosine 1477 and 1487 (Bonazzi et al. 2011). Based on their location within the proximal leg of the triskelion and the fact that dephosphorylation is also required for receptor internalization, we hypothesize that the tyrosine phosphorylation modification might “freeze” clathrin lattices at sites of signaling by preventing addition of further triskelia. CHC17 phosphorylation would thus allow use of clathrin to organize actin locally, as well as synchronize internalization events upon dephosphorylation. In the case of the pathogenic bacteria–host-cell interactions described above, SRC family kinases are required and stabilized clathrin-coated pits are observed at the bacterial–host interface, supporting the idea that bacteria exploit this clathrin modification to organize actin for their own purposes. Observation of clathrin and actin at the T-cell immunological synapse where the TCR signals to phosphorylate clathrin suggests a role for clathrin in local actin organization as well as receptor clearance (Calaibia-Linares et al. 2011).

Sorting Patches Influence Recycling, Degradation, and Metabolism

Purified CHC17 clathrin with or without the CLC will self-assemble into closed polyhedral protein baskets, resembling the coats on clathrin-coated vesicles isolated from brain and other tissues. Electron microscopy (EM) and total internal reflection fluorescence (TIRF) microscopy have revealed that CHC17 clathrin bound to the AP2 adaptor molecule also forms extended flat patches at cell–substrate interfaces (Aggeler and Werb 1982; Saffarian et al. 2009). Additionally, clathrin on sorting endosomes and late endosomes forms nonvesicular patches. On early endosomes, these patches are adjacent to regions of retromer assembly at the edge of tubules (Popoff et al. 2007). It has been suggested that these clathrin patches collect cargo molecules that are then somehow transferred to the retromer-induced tubule. There is, however, no biochemical evidence for interaction between CHC17 and the particular sorting nexins implicated in this pathway (McGough and Cullen 2013). CHC17 is also recruited to endosomes

by ESCRT-0 (Hrs). Hrs-associated CHC17 appears in EM as a double-layered coat (Raiborg et al. 2002), but the details of the molecular contributions to the morphology of this coat have yet to be established. Clathrin does not participate in further steps of MVB formation, which are ESCRT-mediated.

Interaction of CHC22 with sorting nexin 5 (SNX5), a retromer component, was identified by yeast two-hybrid studies and confirmed by mammalian protein expression (Towler et al. 2004). This limited biochemical analysis suggests that CHC22 might interact with retromer at late endosomes in an analogous function to that observed for CHC17 at sorting endosomes. The assembly behavior of CHC22 is still under characterization, and it is not yet known whether CHC22 exerts its function as a sorting patch or as a vesicle coat or both. Nonetheless, its functional role in contributing to formation of the GLUT4 storage compartment (GSC) in skeletal muscle and fat cells is clearly established (Vassilopoulos et al. 2009). The GSC, described elsewhere in this collection (see Antonescu et al. 2014), is a complex network of tubules and vesicles containing the GLUT4 glucose transporter, unique to muscle, fat, and cerebellar neurons (Bryant et al. 2002; Bakirtzi et al. 2009). The GLUT4-containing vesicles and possibly some tubules are released to fuse with the plasma membrane upon insulin signaling, thereby allowing glucose clearance from the blood into the responsive tissues. Depletion of CHC22 prevents GSC formation, whereas CHC22 accumulates on the expanded GSC observed in muscle of insulin-resistant patients with type 2 diabetes (Vassilopoulos et al. 2009). Whether this accumulation contributes to pathogenesis or just reflects the accumulation of sorted cargo is under investigation.

Signaling and Apoptotic Function in Development

Clathrin has recently been implicated in the organization of signalosomes involved in Wnt signaling at the cell surface (Kim et al. 2013). The Wnt signaling pathway is a critical player in development, tissue homeostasis, and metabo-

lism. Wnt binding to its receptors leads to receptor phosphorylation, a process that depends on formation of a signaling patch known as a signalosome. Signalosome formation is dependent on production of PtdIns(4,5)P₂ early in the Wnt signaling cascade. The specificity of the AP2 adaptor for PtdIns(4,5)P₂ causes clathrin accumulation at Wnt signaling sites, and this process was shown to play a role in stabilizing signalosome components at the plasma membrane, without leading to Wnt receptor internalization. Thus, clathrin functions as a scaffold for signalosomes rather than a vehicle for endocytosis.

The phenotype of Hip1-knockout mice suggests a role for Hip1 in late stages of spermatogenesis in the testes (Rao et al. 2001). Hip1 is also involved in forming red blood cells, because mutations of Hip1 lead to hematopoietic abnormalities (Oravec-Wilson et al. 2004). These developmental defects may be a result of Hip1's influence on apoptosis.

Scaffolds for Viral Pathogenesis

HIV exploits several aspects of the endocytic pathway. The Nef protein interacts with both AP1 and AP2 clathrin adaptors, influencing the endocytosis of both class I histocompatibility molecules and CD4 (Roeth et al. 2004; Chaudhuri et al. 2007). The former contributes to immune evasion, and the latter contributes to increased infectivity of HIV particles. Clathrin also plays a nonendocytic role in HIV particle maturation. In this case, clathrin is incorporated into the budding particle as a viral matrix protein and appears to be contributing its function as a scaffold to organize viral core proteins (Zhang et al. 2011).

Enveloped viruses engulf themselves with patches of the cellular membrane as they assemble and subsequently bud into either endosome-like compartments or from the plasma membrane, the location varying for different viruses and different cell types (Welsch et al. 2007; Benaroch et al. 2010). For these budding pathways, most enveloped viruses, including HIV, co-opt ESCRT proteins normally used by cells to generate multivesicular bodies (MVBs)

from endosomes. Although MVB formation requires the sequential action of ESCRT-0 and then ESCRT-I–III, enveloped viruses use their own proteins to substitute for the ESCRT-0, by directly linking the ESCRT-I protein TSG101 to the viral GAG protein, and also bypass ESCRT-II by using the adaptor protein Alix to link ESCRT-III to GAG (Pornillos et al. 2003; Langelier et al. 2006; Medina et al. 2011). Although ESCRT-II may not be used in viral budding (Langelier et al. 2006), a role for ESCRT-II proteins was shown in other aspects of HIV infection. siRNA-mediated depletion of the ESCRT-II protein EAP30 blocked the normal trafficking of HIV-1 genomic RNA and resulted in an accumulation of genomic HIV-1 RNA in the nucleus and juxtanuclear domains (Ghoujal et al. 2012). In this case, it would appear that ESCRT-II is playing a nonendocytic role in the HIV life cycle.

Cell Division

Temporal control of clathrin inactivation in synchronized cells showed that the interaction of the terminal domain of CHC17 with the TACC3–chTOG complex at the onset of S phase influences both centrosome and spindle integrity during the cell cycle. If CHC17 is inactivated at S phase by drug-induced cross-linking of CLC, centrosome maturation is affected during the onset of mitosis, resulting in fragmented centrosomes occur and multipolar spindles (Foraker et al. 2012). chTOG localization to the centrosome was decreased upon clathrin inactivation, and because chTOG is required for centrosome maturation (Cassimeris and Morabito 2004), clathrin inactivation could possibly affect centrosome integrity by failing to complex with chTOG. Spindle defects also occur upon clathrin depletion and are attributed to direct binding of clathrin to kinetochore microtubules via the TACC3–chTOG complex (Booth et al. 2011). Loss of clathrin from the spindle consequently induces chromosome dysjunction (Royle et al. 2005).

Clathrin and associated proteins also play a role in cytokinesis, the last step of cell division. This role for clathrin is observed in a wide

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variety of organisms from protists to plant cells to animal cells despite widely different mechanisms of cell division in these cells. In plants, cell division is accomplished by building a cell wall between the two daughter cells. Here the pre-prophase band and the phragmoplast, the locations where the cell plate will fuse together, are sites of abundant clathrin activity (Jurgens 2005; Tahara et al. 2007; Karahara et al. 2009). In animal cells, an actomyosin contractile ring that constricts at the equator facilitates cell division (Pollard 2010). Live-cell imaging has shown that after anaphase, GFP-labeled clathrin coated structures move toward the equator and disappear, suggesting a role for clathrin in remodeling the plasma membrane at the equator (Warner et al. 2006). It is not known precisely what essential role these clathrin-coated structures play, but live-cell imaging in *Dictyostelium* cells suggests that this event is late in cytokinesis, after constriction of the actomyosin ring (Gerald et al. 2001). A role late in cytokinesis for clathrin and associated proteins was confirmed recently in animal cells by showing that siRNA depletion of several endocytic proteins blocked completion of abscission but not constriction of the cleavage furrow in dividing cells (Smith and Chircop 2012). Additionally, dynamin plays a role in abscission (Joshi et al. 2010). Because of their ability to capture membrane and specific proteins and lipids, clathrin-coated structures could coordinate the massive remodeling at the equator of either plant or animal cells and thereby establish the essential membrane or protein composition needed to complete cytokinesis. Several lines of evidence indicate that rab11 recycling endosomes play crucial roles in supplying membrane at the cleavage furrow late in cytokinesis (Wilson et al. 2005). Genetic and other evidence has implicated many other membrane trafficking proteins as key players in cytokinesis (McKay and Burgess 2011).

During late stages of cytokinesis, proteins in the ESCRT-III complex are recruited to the midbody complex. Here they are thought to assist in the severing of membranes in the intercellular bridge that connects the daughter cells, analogous to their role in assisting fusion in the formation of MVBs or in budding of envel-

oped viruses, although the intercellular bridge that occurs during cytokinesis requires fusion across a larger distance than occurs in MVB or a virus budding (Fig. 2). Recent high-resolution, live-cell imaging of the bridge in dividing cells coupled with electron tomography shows that filaments consistent with the size of filaments made by ESCRT-III proteins could drive this process and also provide a scaffold to recruit proteins required for the removal of the midbody (Guizetti et al. 2011). Recent evidence suggests that ESCRT-III complexes could also assist in regulating the final stages of cytokinesis. Members of the ESCRT-III complex promote the Aurora B abscission checkpoint (Carlton et al. 2012), a cell regulation role that could occur independently of the fusion function typically associated with ESCRT complexes.

Transcriptional Function and Oncogenesis

Loss of p53 is associated with oncogenesis because p53 activation controls genes for growth arrest or apoptosis in response to DNA damage. Clathrin heavy chain has been shown to be a p53 activator (Enari et al. 2006). The exogenous expression of CHC17 increases the transactivation of p53-responsive promoters (*p53AIP1*, *P21^{waf1}*, *p53R2*, and *Noxa*), whereas RNAi silencing reduces p53 transcriptional activity (Enari et al. 2006). There are data showing that CHC17 recruits histone acetyltransferase p300 to p53 to promote p53-mediated transcription (Enari et al. 2006; Ohmori et al. 2008). Nuclear fractionation and immuno-electron microscopy studies detect a small fraction (~5%) of CHC17 in the nucleus (Enari et al. 2006). Luciferase-based reporter assays indicate that trimeric clathrin is not required to participate in p53-mediated transcription and that there is increased nuclear localization when the trimerization domain is artificially deleted (Ohmori et al. 2008). However, this begs the question: How does clathrin for the nucleus detrimmerize in the cytosol? The detrimmerization switch hypothesis offers a way to understand how heavy-chain legs of trimeric clathrin can be detrimmerized without removing the trimeri-

zation domain (Fig. 1). CHC17 clathrin hub (amino acids 1074–1675) is reduced to a mixture of nontrimeric molecules when cysteine-1573—located in the middle of mobile Helix 7j—is mutated to alanine (Ybe et al. 2013). These nontrimeric CHC17 hub molecules localize to the nuclei of a variety of human cancer cell lines (Ybe et al. 2013). It is not yet known what, if any, role CLC plays in dislocating clathrin heavy-chain legs for nuclear translocation. There is some evidence suggesting that CLC is involved because in vitro studies show that the carboxyl terminus of CLC contributes to the stability of clathrin triskelion (Ybe et al. 2003, 2007b, 2013). The proposed detrimerization switch (Fig. 1) has potential implications for oncogenesis if further studies continue to support a connection between monomeric clathrin and p53 function.

Hip1R was the first human homolog of Sla2p to be identified as a cofactor in clathrin-mediated endocytosis (CME) (Engqvist-Goldstein et al. 1999, 2001). Like its relative, Hip1 is also a player in CME (Mishra et al. 2001; Rao et al. 2001; Waelter et al. 2001), but is functionally distinct from Hip1R because Hip1 is involved in human cancer. Analysis of tissue samples revealed that Hip1 is a consistent marker for solid tumors, with elevated levels in cell lines derived from breast, colon, kidney, lung, melanoma, ovarian, and prostate tumors (Rao et al. 2001; Hyun and Ross 2004). It was observed that the overexpression of Hip1 is correlated to the progression of prostate cancer (Rao et al. 2001). As the cancer progresses, there are changes in the transcriptional response and expression of androgen receptor (AR) (Chen et al. 2004). AR is a member of the nuclear hormone receptor superfamily of transcription factors and has polyglutamine and polyglycine repeats in the amino-terminal domain, which interact with transcriptional coregulators. The polyglutamine tract in AR may also facilitate its binding to Hip1, which can interact with proteins with polyglutamine repeats. Hip1 binds to AR through its central coiled-coil domain, and stimulation by androgen causes Hip1 to be recruited to DNA response elements (Mills et al. 2005). AR-mediated transcription is di-

minished when Hip1 expression is lowered and activated when Hip1 is overexpressed (Mills et al. 2005). Hip1 has a functional, but weak nuclear localization signal (NLS) in its carboxyl terminus (Mills et al. 2005). The activities of Hip1 in the nucleus link this endocytic protein to the transcriptional modulation of hormone-responsive genes. Furthermore, the ability of Hip1 to be in the nucleus raises the possibility that nuclear clathrin could associate with Hip1 to mediate nonendocytic cellular events that we do not yet know about. Hip1 may also influence oncogenesis via signaling. In chronic myelomonocytic leukemia, a gene translocation fuses Hip1 to platelet-derived growth factor β receptor (PDGFR β) (Ross et al. 1998).

The endocytic role of the clathrin adaptor Numb and the importance of Numb in regulation of the Notch signaling pathway in developing embryos have been extensively characterized. However, recent studies show an additional role for Numb as a tumor suppressor independent of its role in endocytosis and Notch regulation. Numb binds to both p53 and the E3 ubiquitin ligase Mdm 2, which is known to shuttle Numb into the nucleus and is a potent regulator of the tumor suppressor p53 (Jung-Gershon et al. 1998; Colaluca et al. 2008). Additionally, Numb forms dimeric and trimeric complexes with Mdm2 and p53, thereby inhibiting degradation of p53 (Colaluca et al. 2008). Therefore, loss of Numb can result in increased cell proliferation by two separate means: (1) an increase in Notch signaling, and (2) attenuation of p53 signaling. The biological importance of Numb signaling is underscored by the observation that about half of human breast carcinomas display a loss of Numb-mediated control of Notch signaling (Pece et al. 2004).

Although the APPLs normally function as Rab5 effectors in endosomal trafficking, extracellular stimuli such as oxidative stress or growth factor internalization promotes GTP hydrolysis by rab5, resulting in release of the APPL proteins from endosomal membranes into the cytosol and translocation of the APPLs into the nucleus. Once in the nucleus, both APPLs interact with the nucleosome remodeling and HDAC (histone deacetylase) multi-

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protein complex, NuRD/MeCP1, suggesting a role for the APPL proteins in transcriptional regulation. More recent studies provide direct evidence for transcriptional regulation by the APPL proteins. Nuclear-localized APPL proteins stimulate β -catenin/TCF-dependent transcription (Rashid et al. 2009). Here, shuttling of APPLs into the nucleus results in dissociation of HDAC1, HDAC2, and β -catenin from the transcriptional repressor Reptin. Taken together, these studies reveal an important link between endocytic trafficking and signal-induced transcriptional activation.

Fusion of endocytic genes with other genes can lead to new roles. Chromosomal translocation and fusion of the CALM gene with the AF10 gene is associated with acute myeloid leukemia, suggesting a possible link between endocytic defects and oncogenesis (Dreyling et al. 1998; Kumon et al. 1999; Narita et al. 1999). However, recent studies suggest that the contribution of CALM to CALM–AF10-mediated oncogenic transformation is independent of disruption of endocytosis (Stoddart et al. 2012). Rather, evidence suggests that the fusion with CALM leads to the oligomerization of CALM–AF10, potentially influencing transcriptional regulation leading to myeloid leukemias. Additional studies have found that CALM–AF10 contributes to up-regulation of several DNA repair and maintenance genes (*Commd3*, *Bmi1*, *Dnajc1*, and *Spag6*) (Mulaw et al. 2012). The functional consequences of this up-regulation have yet to be determined.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Here we describe multiple nonendocytic functions for endocytosis regulators that impact cytoskeleton organization, cell division, and gene regulation, as well as nonendocytic sorting pathways. From an evolutionary perspective, some of these nonconventional functions have emerged as recent specializations from gene duplication, and others likely represent residual ancient functions of endocytic proteins. Some result from splice variants, and some are generated as a result of novel posttranslational mod-

ification. It is likely that the current and future forays in whole-genome screening and analysis of “interactomes” will reveal further unexpected roles for endocytosis regulators in nonendocytic pathways. The known “extra functions” already influence pathways of infection, oncogenesis, and glucose metabolism, showing the value of keeping an open mind about the functions of endocytic regulators and their relevance to human health and disease.

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REFERENCES

* Reference is also in this collection.

- Aggeler J, Werb Z. 1982. Initial events during phagocytosis by macrophages viewed from outside and inside the cell: Membrane-particle interactions and clathrin. *J Cell Biol* **94**: 613–623.
- Aghamohammadzadeh S, Ayscough KR. 2009. Differential requirements for actin during yeast and mammalian endocytosis. *Nat Cell Biol* **11**: 1039–1042.
- Ahle S, Ungewickell E. 1986. Purification and properties of a new clathrin assembly protein. *EMBO J* **5**: 3143–3149.
- * Antonescu CN, McGraw TE, Klip A. 2014. Reciprocal regulation of endocytosis and metabolism. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a016964.
- Archangelo LE, Glasner J, Krause A, Bohlander SK. 2006. The novel CALM interactor CATS influences the subcellular localization of the leukemogenic fusion protein CALM/AF10. *Oncogene* **25**: 4099–4109.
- Bakirtzi K, Belfort G, Lopez-Coviella I, Kuruppu D, Cao L, Abel ED, Brownell AL, Kandror KV. 2009. Cerebellar neurons possess a vesicular compartment structurally and functionally similar to Glut4-storage vesicles from peripheral insulin-sensitive tissues. *J Neurosci* **29**: 5193–5201.
- Benaroch P, Billard E, Gaudin R, Schindler M, Jouve M. 2010. HIV-1 assembly in macrophages. *Retrovirology* **7**: 29.
- Benmerah A. 2004. Endocytosis: Signaling from endocytic membranes to the nucleus. *Curr Biol* **14**: R314–R316.
- Berdnik D, Torok T, Gonzalez-Gaitan M, Knoblich JA. 2002. The endocytic protein α -adaptin is required for Numb-mediated asymmetric cell division in *Drosophila*. *Dev Cell* **3**: 221–231.

Unconventional Functions of Endocytosis Regulators

- * Bökel C, Brand M. 2014. Endocytosis and signaling during development. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a017020.
- Bonazzi M, Vasudevan L, Mallet A, Sachse M, Sartori A, Prevost MC, Roberts A, Taner SB, Wilbur JD, Brodsky FM, et al. 2011. Clathrin phosphorylation is required for actin recruitment at sites of bacterial adhesion and internalization. *J Cell Biol* **195**: 525–536.
- Bonazzi M, Kuhbacher A, Toledo-Arana A, Mallet A, Vasudevan L, Pizarro-Cerda J, Brodsky FM, Cossart P. 2012. A common clathrin-mediated machinery co-ordinates cell-cell adhesion and bacterial internalization. *Traffic* **13**: 1653–1666.
- Booth DG, Hood FE, Prior IA, Royle SJ. 2011. A TACC3/chTOG/clathrin complex stabilises kinetochore fibres by inter-microtubule bridging. *EMBO J* **30**: 906–919.
- Boulant S, Kural C, Zeeh JC, Ubelmann F, Kirchhausen T. 2011. Actin dynamics counteract membrane tension during clathrin-mediated endocytosis. *Nat Cell Biol* **13**: 1124–1131.
- Brett TJ, Traub LM, Fremont DH. 2002. Accessory protein recruitment motifs in clathrin-mediated endocytosis. *Structure* **10**: 797–809.
- Brett TJ, Legendre-Guillemain V, McPherson PS, Fremont DH. 2006. Structural definition of the F-actin-binding THATCH domain from HIP1R. *Nat Struct Mol Biol* **13**: 121–130.
- Brodsky FM. 2012. Diversity of clathrin function: New tricks for an old protein. *Annu Rev Cell Dev Biol* **28**: 309–336.
- Bryant NJ, Govers R, James DE. 2002. Regulated transport of the glucose transporter GLUT4. *Nat Rev Mol Cell Biol* **3**: 267–277.
- Calabia-Linares C, Robles-Valero J, de la Fuente H, Perez-Martinez M, Martin-Cofreces N, Alfonso-Perez M, Gutierrez-Vazquez C, Mittelbrunn M, Ibiza S, Urbano-Olmos FR, et al. 2011. Endosomal clathrin drives actin accumulation at the immunological synapse. *J Cell Sci* **124**: 820–830.
- Carlton JG, Caballe A, Agromayor M, Kloc M, Martin-Serrano J. 2012. ESCRT-III governs the Aurora B-mediated abscission checkpoint through CHMP4C. *Science* **336**: 220–225.
- Cassimeris L, Morabito J. 2004. TOGp, the human homolog of XMAP215/Dis1, is required for centrosome integrity, spindle pole organization, and bipolar spindle assembly. *Mol Biol Cell* **15**: 1580–1590.
- Chaudhuri R, Lindwasser OW, Smith WJ, Hurley JH, Bonifacio JS. 2007. Downregulation of CD4 by human immunodeficiency virus type 1 Nef is dependent on clathrin and involves direct interaction of Nef with the AP2 clathrin adaptor. *J Virol* **81**: 3877–3890.
- Cheeseman LP, Booth DG, Hood FE, Prior IA, Royle SJ. 2011. Aurora A kinase activity is required for localization of TACC3/ch-TOG/clathrin inter-microtubule bridges. *Commun Integr Biol* **4**: 409–412.
- Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG, Sawyers CL. 2004. Molecular determinants of resistance to anti-androgen therapy. *Nat Med* **10**: 33–39.
- Chopra VS, Metzler M, Rasper DM, Engqvist-Goldstein AE, Singaraja R, Gan L, Fichter KM, McCutcheon K, Drubin D, Nicholson DW, et al. 2000. HIP12 is a non-proapoptotic member of a gene family including HIP1, an interacting protein with huntingtin. *Mamm Genome* **11**: 1006–1015.
- Chu DS, Pishvaei B, Payne GS. 1996. The light chain subunit is required for clathrin function in *Saccharomyces cerevisiae*. *J Biol Chem* **271**: 33123–33130.
- Colaluca IN, Tosoni D, Nuciforo P, Senic-Matuglia F, Galimberti V, Viale G, Pece S, Di Fiore PP. 2008. NUMB controls p53 tumour suppressor activity. *Nature* **451**: 76–80.
- * Cosker KE, Segal RA. 2014. Neuronal signaling through endocytosis. *Cold Spring Harb Perspect Biol* **6**: a020669.
- * Cossart P, Helenius A. 2014. Endocytosis of viruses and bacteria. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a016972.
- Crotzer VL, Mabardy AS, Weiss A, Brodsky FM. 2004. T cell receptor engagement leads to phosphorylation of clathrin heavy chain during receptor internalization. *J Exp Med* **199**: 981–991.
- * Di Fiore PP, von Zastrow M. 2014. Endocytosis, signaling and beyond. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a016865.
- Dreyling MH, Schrader K, Fonatsch C, Schlegelberger B, Haase D, Schoch C, Ludwig W, Löffler H, Buchner T, Wormann B, et al. 1998. MLL and CALM are fused to AF10 in morphologically distinct subsets of acute leukemia with translocation t(10;11): Both rearrangements are associated with a poor prognosis. *Blood* **91**: 4662–4667.
- * Eaton S, Martin-Belmonte F. 2014. Cargo sorting in the endocytic pathway: A key regulator of cell polarity and tissue dynamics. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a016899.
- Enari M, Ohmori K, Kitabayashi I, Taya Y. 2006. Requirement of clathrin heavy chain for p53-mediated transcription. *Genes Dev* **20**: 1087–1099.
- Engqvist-Goldstein AE, Kessels MM, Chopra VS, Hayden MR, Drubin DG. 1999. An actin-binding protein of the Sla2/huntingtin interacting protein 1 family is a novel component of clathrin-coated pits and vesicles. *J Cell Biol* **147**: 1503–1518.
- Engqvist-Goldstein AE, Warren RA, Kessels MM, Keen JH, Heuser J, Drubin DG. 2001. The actin-binding protein Hip1R associates with clathrin during early stages of endocytosis and promotes clathrin assembly in vitro. *J Cell Biol* **154**: 1209–1223.
- Esk C, Chen CY, Johannes L, Brodsky FM. 2010. The clathrin heavy chain isoform CHC22 functions in a novel endosomal sorting step. *J Cell Biol* **188**: 131–144.
- Fontaine SN, Bauer SP, Lin X, Poorfarahani S, Ybe JA. 2012. Replacement of charged and polar residues in the coiled-coiled interface of huntingtin-interacting protein 1 (HIP1) causes aggregation and cell death. *FEBS Lett* **586**: 3030–3036.
- Foraker AB, Camus SM, Evans TM, Majeed SR, Chen CY, Taner SB, Correa IR Jr, Doxsey SJ, Brodsky FM. 2012. Clathrin promotes centrosome integrity in early mitosis through stabilization of centrosomal ch-TOG. *J Cell Biol* **198**: 591–605.

F.M. Brodsky et al.

- Ford MG, Pearse BM, Higgins MK, Vallis Y, Owen DJ, Gibson A, Hopkins CR, Evans PR, McMahon HT. 2001. Simultaneous binding of PtdIns(4,5)P₂ and clathrin by AP180 in the nucleation of clathrin lattices on membranes. *Science* **291**: 1051–1055.
- Fotin A, Cheng Y, Sliz P, Grigorieff N, Harrison SC, Kirchhausen T, Walz T. 2004. Molecular model for a complete clathrin lattice from electron cryomicroscopy. *Nature* **432**: 573–579.
- Gerald NJ, Damer CK, O'Halloran TJ, De Lozanne A. 2001. Cytokinesis failure in clathrin-minus cells is caused by cleavage furrow instability. *Cell Motil Cytoskeleton* **48**: 213–223.
- Gervais FG, Singaraja R, Xanthoudakis S, Gutekunst CA, Leavitt BR, Metzler M, Hackam AS, Tam J, Vaillancourt JP, Houtzager V, et al. 2002. Recruitment and activation of caspase-8 by the huntingtin-interacting protein Hip-1 and a novel partner Hippi. *Nat Cell Biol* **4**: 95–105.
- Ghoujal B, Milev MP, Ajamian L, Abel K, Moulant AJ. 2012. ESCRT-II's involvement in HIV-1 genomic RNA trafficking and assembly. *Biol Cell* **104**: 706–721.
- * Gonzalez-Gaitan M, Jülicher F. 2014. The role of endocytosis during morphogenetic signaling. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a016881.
- Guizetti J, Schermelleh L, Mantler J, Maar S, Poser I, Leonhardt H, Muller-Reichert T, Gerlich DW. 2011. Cortical constriction during abscission involves helices of ESCRT-III-dependent filaments. *Science* **331**: 1616–1620.
- Guo M, Jan LY, Jan YN. 1996. Control of daughter cell fates during asymmetric division: Interaction of Numb and Notch. *Neuron* **17**: 27–41.
- Hackam AS, Yassa AS, Singaraja R, Metzler M, Gutekunst CA, Gan L, Warby S, Wellington CL, Vaillancourt J, Chen N, et al. 2000. Huntingtin interacting protein 1 induces apoptosis via a novel caspase-dependent death effector domain. *J Biol Chem* **275**: 41299–41308.
- Henne WM, Buchkovich NJ, Emr SD. 2011. The ESCRT pathway. *Dev Cell* **21**: 77–91.
- * Henne WM, Stenmark H, Emr SD. 2013. Molecular mechanisms of the membrane sculpting ESCRT pathway. *Cold Spring Harb Perspect Biol* **5**: a016766.
- Huang KM, Gullberg L, Nelson KK, Stefan CJ, Blumer K, Lemmon SK. 1997. Novel functions of clathrin light chains: Clathrin heavy chain trimerization is defective in light chain-deficient yeast. *J Cell Sci* **110**: 899–910.
- Huang F, Khvorova A, Marshall W, Sorkin A. 2004. Analysis of clathrin-mediated endocytosis of epidermal growth factor receptor by RNA interference. *J Biol Chem* **279**: 16657–16661.
- Hurley JH. 2010. The ESCRT complexes. *Crit Rev Biochem Mol Biol* **45**: 463–487.
- Hyun TS, Ross TS. 2004. HIP1: Trafficking roles and regulation of tumorigenesis. *Trends Mol Med* **10**: 194–199.
- Joshi S, Perera S, Gilbert J, Smith CM, Mariana A, Gordon CP, Sakoff JA, McCluskey A, Robinson PJ, Braithwaite AW, et al. 2010. The dynamin inhibitors MiTMAB and OcTMAB induce cytokinesis failure and inhibit cell proliferation in human cancer cells. *Mol Cancer Ther* **9**: 1995–2006.
- Jurgens G. 2005. Cytokinesis in higher plants. *Annu Rev Plant Biol* **56**: 281–299.
- Juven-Gershon T, Shifman O, Unger T, Elkeles A, Haupt Y, Oren M. 1998. The Mdm2 oncoprotein interacts with the cell fate regulator Numb. *Mol Cell Biol* **18**: 3974–3982.
- Kalchman MA, Koide HB, McCutcheon K, Graham RK, Nichol K, Nishiyama K, Kazemi-Esfarjani P, Lynn FC, Wellington C, Metzler M, et al. 1997. HIP1, a human homologue of *S. cerevisiae* Sla2p, interacts with membrane-associated huntingtin in the brain. *Nat Genet* **16**: 44–53.
- Karahara I, Suda J, Tahara H, Yokota E, Shimmen T, Misaki K, Yonemura S, Staehelin LA, Mineyuki Y. 2009. The preprophase band is a localized center of clathrin-mediated endocytosis in late prophase cells of the onion cotyledon epidermis. *Plant J* **57**: 819–831.
- Kim I, Pan W, Jones SA, Zhang Y, Zhuang X, Wu D. 2013. Clathrin and AP2 are required for PtdIns(4,5)P₂-mediated formation of LRP6 signalosomes. *J Cell Biol* **200**: 419–428.
- * Kirchhausen T, Owen D, Harrison SC. 2014. Molecular structure, function and dynamics of clathrin-mediated membrane traffic. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a016725.
- Kumon K, Kobayashi H, Maseki N, Sakashita A, Sakurai M, Tanizawa A, Imashuku S, Kaneko Y. 1999. Mixed-lineage leukemia with t(10;11)(p13;q21): An analysis of AF10-CALM and CALM-AF10 fusion mRNAs and clinical features. *Genes Chromosomes Cancer* **25**: 33–39.
- Langelier C, von Schwedler UK, Fisher RD, De Domenico I, White PL, Hill CP, Kaplan J, Ward D, Sundquist WI. 2006. Human ESCRT-II complex and its role in human immunodeficiency virus type 1 release. *J Virol* **80**: 9465–9480.
- Legendre-Guillemin V, Wasiak S, Hussain NK, Angers A, McPherson PS. 2004. ENTH/ANTH proteins and clathrin-mediated membrane budding. *J Cell Sci* **117**: 9–18.
- Legendre-Guillemin V, Metzler M, Lemaire JF, Philie J, Gan L, Hayden MR, McPherson PS. 2005. Huntingtin interacting protein 1 (HIP1) regulates clathrin assembly through direct binding to the regulatory region of the clathrin light chain. *J Biol Chem* **280**: 6101–6108.
- Liu SH, Towler MC, Chen E, Chen CY, Song W, Apodaca G, Brodsky FM. 2001. A novel clathrin homolog that co-distributes with cytoskeletal components functions in the trans-Golgi network. *EMBO J* **20**: 272–284.
- * Mayor S, Parton RG, Donaldson JG. 2014. Clathrin-independent pathways of endocytosis. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a016758.
- McDonald B, Martin-Serrano J. 2009. No strings attached: The ESCRT machinery in viral budding and cytokinesis. *J Cell Sci* **122**: 2167–2177.
- McGough IJ, Cullen PJ. 2013. Clathrin is not required for SNX-BAR-retromer-mediated carrier formation. *J Cell Sci* **126**: 45–52.
- McKay HE, Burgess DR. 2011. "Life is a highway": Membrane trafficking during cytokinesis. *Traffic* **12**: 247–251.
- Medina GN, Ehrlich LS, Chen MH, Khan MB, Powell MD, Carter CA. 2011. Sprouty 2 binds ESCRT-II factor Eap20 and facilitates HIV-1 gag release. *J Virol* **85**: 7353–7362.
- * Mellman I, Yarden Y. 2013. Endocytosis and cancer. *Cold Spring Harb Perspect Biol* **5**: a016949.

Unconventional Functions of Endocytosis Regulators

- * Merrifield CJ, Kaksonen M. 2014. Endocytic accessory factors and regulation of clathrin-mediated endocytosis. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a016733.
- Miaczynska M, Christoforidis S, Giner A, Shevchenko A, Uttenweiler-Joseph S, Habermann B, Wilm M, Parton RG, Zerial M. 2004. APPL proteins link Rab5 to nuclear signal transduction via an endosomal compartment. *Cell* **116**: 445–456.
- Mills IG, Gaughan L, Robson C, Ross T, McCracken S, Kelly J, Neal DE. 2005. Huntingtin interacting protein 1 modulates the transcriptional activity of nuclear hormone receptors. *J Cell Biol* **170**: 191–200.
- Mishra SK, Agostinelli NR, Brett TJ, Mizukami I, Ross TS, Traub LM. 2001. Clathrin- and AP-2-binding sites in HIP1 uncover a general assembly role for endocytic accessory proteins. *J Biol Chem* **276**: 46230–46236.
- * Morgan JR, Comstra HS, Cohen M, Faundez V. 2013. Presynaptic membrane retrieval and endosome biology: Defining molecularly heterogeneous synaptic vesicles. *Cold Spring Harb Perspect Biol* **5**: a016915.
- Morita E. 2012. Differential requirements of mammalian ESCRTs in multivesicular body formation, virus budding and cell division. *FEBS J* **279**: 1399–1406.
- Mulaw MA, Krause A, Deshpande AJ, Krause LE, Rouhi A, La Starza R, Borkhardt A, Buske C, Mecucci C, Ludwig WD, et al. 2012. CALM/AF10-positive leukemias show upregulation of genes involved in chromatin assembly and DNA repair processes and of genes adjacent to the breakpoint at 10p12. *Leukemia* **26**: 1012–1019.
- Narita M, Shimizu K, Hayashi Y, Taki T, Taniwaki M, Hosoda F, Kobayashi H, Nakamura H, Sadamori N, Ohnishi H, et al. 1999. Consistent detection of CALM-AF10 chimeric transcripts in haematological malignancies with t(10;11)(p13;q14) and identification of novel transcripts. *Br J Haematol* **105**: 928–937.
- Niu Q, Ybe JA. 2008. Crystal structure at 2.8 Å of huntingtin-interacting protein 1 (HIP1) coiled-coil domain reveals a charged surface suitable for HIP1 protein interactor (HIPPI). *J Mol Biol* **375**: 1197–1205.
- Ohmori K, Endo Y, Yoshida Y, Ohata H, Taya Y, Enari M. 2008. Monomeric but not trimeric clathrin heavy chain regulates p53-mediated transcription. *Oncogene* **27**: 2215–2227.
- Oravec-Wilson KI, Kiel MJ, Li L, Rao DS, Saint-Dic D, Kumar PD, Provot MM, Hankenson KD, Reddy VN, Lieberman AP, et al. 2004. Huntingtin Interacting Protein 1 mutations lead to abnormal hematopoiesis, spinal defects and cataracts. *Hum Mol Genet* **13**: 851–867.
- Pearse BMF. 1975. Coated vesicles from pig brain: Purification and biochemical characterization. *J Mol Biol* **97**: 93–98.
- Pece S, Serresi M, Santolini E, Capra M, Hulleman E, Galimberti V, Zurrida S, Maisonneuve P, Viale G, Di Fiore PP. 2004. Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis. *J Cell Biol* **167**: 215–221.
- Peel S, Macheboeuf P, Martinelli N, Weissenhorn W. 2011. Divergent pathways lead to ESCRT-III-catalyzed membrane fission. *Trends Biochem Sci* **36**: 199–210.
- Pilecka I, Banach-Orlowska M, Miaczynska M. 2007. Nuclear functions of endocytic proteins. *Eur J Cell Biol* **86**: 533–547.
- Pollard TD. 2010. Mechanics of cytokinesis in eukaryotes. *Curr Opin Cell Biol* **22**: 50–56.
- Popoff V, Mardones GA, Tenza D, Rojas R, Lamaze C, Bonifacio JS, Raposo G, Johannes L. 2007. The retromer complex and clathrin define an early endosomal retrograde exit site. *J Cell Sci* **120**: 2022–2031.
- Pornillos O, Higginson DS, Stray KM, Fisher RD, Garrus JE, Payne M, He GP, Wang HE, Morham SG, Sundquist WI. 2003. HIV Gag mimics the Tsg101-recruiting activity of the human Hrs protein. *J Cell Biol* **162**: 425–434.
- Raiborg C, Bache KG, Gillooly DJ, Madhus IH, Stang E, Stenmark H. 2002. Hrs sorts ubiquitinated proteins into clathrin-coated microdomains of early endosomes. *Nat Cell Biol* **4**: 394–398.
- Rao DS, Chang JC, Kumar PD, Mizukami I, Smithson GM, Bradley SV, Parlow AF, Ross TS. 2001. Huntingtin interacting protein 1 is a clathrin coat binding protein required for differentiation of late spermatogenic progenitors. *Mol Cell Biol* **21**: 7796–7806.
- Rashid S, Pilecka I, Torun A, Olchowik M, Bielinska B, Miaczynska M. 2009. Endosomal adaptor proteins APPL1 and APPL2 are novel activators of β -catenin/TCF-mediated transcription. *J Biol Chem* **284**: 18115–18128.
- Roeth JF, Williams M, Kasper MR, Filzen TM, Collins KL. 2004. HIV-1 Nef disrupts MHC-I trafficking by recruiting AP-1 to the MHC-I cytoplasmic tail. *J Cell Biol* **167**: 903–913.
- Ross TS, Bernard OA, Berger R, Gilliland DG. 1998. Fusion of huntingtin interacting protein 1 to platelet-derived growth factor β receptor (PDGFR) in chronic myelomonocytic leukemia with t(5;7)(q33;q11.2). *Blood* **91**: 4419–4426.
- Royle SJ. 2012. The role of clathrin in mitotic spindle organisation. *J Cell Sci* **125**: 19–28.
- Royle SJ, Bright NA, Lagnado L. 2005. Clathrin is required for the function of the mitotic spindle. *Nature* **434**: 1152–1157.
- Saffarian S, Cocucci E, Kirchhausen T. 2009. Distinct dynamics of endocytic clathrin-coated pits and coated plaques. *PLoS Biol* **7**: e1000191.
- Santolini E, Puri C, Salcini AE, Gagliani MC, Pelicci PG, Tacchetti C, Di Fiore PP. 2000. Numb is an endocytic protein. *J Cell Biol* **151**: 1345–1352.
- Scita G, Di Fiore PP. 2010. The endocytic matrix. *Nature* **463**: 464–473.
- Smith CM, Chircop M. 2012. Clathrin-mediated endocytic proteins are involved in regulating mitotic progression and completion. *Traffic* **13**: 1628–1641.
- Stavrou I, O'Halloran TJ. 2006. The monomeric clathrin assembly protein, AP180, regulates contractile vacuole size in *Dictyostelium discoideum*. *Mol Biol Cell* **17**: 5381–5389.
- Stoddart A, Dykstra ML, Brown BK, Song W, Pierce SK, Brodsky FM. 2002. Lipid rafts unite signaling cascades with clathrin to regulate BCR internalization. *Immunity* **17**: 451–462.

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- Stoddart A, Tennant TR, Fernald AA, Anastasi J, Brodsky FM, Le Beau MM. 2012. The clathrin-binding domain of CALM-AF10 alters the phenotype of myeloid neoplasms in mice. *Oncogene* **31**: 494–506.
- Tahara H, Yokota E, Igarashi H, Orii H, Yao M, Sonobe S, Hashimoto T, Hussey PJ, Shimmen T. 2007. Clathrin is involved in organization of mitotic spindle and phragmoplast as well as in endocytosis in tobacco cell cultures. *Protoplasma* **230**: 1–11.
- Tebar F, Bohlander SK, Sorkin A. 1999. Clathrin assembly lymphoid myeloid leukemia (CALM) protein: Localization in endocytic-coated pits, interactions with clathrin, and the impact of overexpression on clathrin-mediated traffic. *Mol Biol Cell* **10**: 2687–2702.
- * ten Broeke T, Wubbolts R, Stoorvogel W. 2013. MHC class II antigen presentation by dendritic cells regulated through endosomal sorting. *Cold Spring Harb Perspect Biol* **5**: a016873.
- Towler MC, Gleeson PA, Hoshino S, Rahkila P, Manalo V, Ohkoshi N, Ordahl C, Parton RG, Brodsky FM. 2004. Clathrin isoform CHC22, a component of neuromuscular and myotendinous junctions, binds sorting nexin 5 and has increased expression during myogenesis and muscle regeneration. *Mol Biol Cell* **15**: 3181–3195.
- Vassilopoulos S, Esk C, Hoshino S, Funke BH, Chen CY, Plocik AM, Wright WE, Kucherlapati R, Brodsky FM. 2009. A role for the CHC22 clathrin heavy-chain isoform in human glucose metabolism. *Science* **324**: 1192–1196.
- Vecchi M, Polo S, Poupon V, van de Loo JW, Benmerah A, Di Fiore PP. 2001. Nucleocytoplasmic shuttling of endocytic proteins. *J Cell Biol* **153**: 1511–1517.
- Waelter S, Scherzinger E, Hasenbank R, Nordhoff E, Lurz R, Goehler H, Gauss C, Sathasivam K, Bates GP, Lehrach H, et al. 2001. The huntingtin interacting protein HIP1 is a clathrin and α -adaptin-binding protein involved in receptor-mediated endocytosis. *Hum Mol Genet* **10**: 1807–1817.
- Wang J, Virta VC, Riddelle-Spencer K, O'Halloran TJ. 2003. Compromise of clathrin function and membrane association by clathrin light chain deletion. *Traffic* **4**: 891–901.
- Wanker EE, Rovira C, Scherzinger E, Hasenbank R, Walter S, Tait D, Colicelli J, Lehrach H. 1997. HIP-I: A huntingtin interacting protein isolated by the yeast two-hybrid system. *Hum Mol Genet* **6**: 487–495.
- Warner AK, Keen JH, Wang YL. 2006. Dynamics of membrane clathrin-coated structures during cytokinesis. *Traffic* **7**: 205–215.
- Welsch S, Muller B, Krausslich HG. 2007. More than one door—Budding of enveloped viruses through cellular membranes. *FEBS Lett* **581**: 2089–2097.
- Wilbur JD, Hwang PK, Brodsky FM, Fletterick RJ. 2010a. Accommodation of structural rearrangements in the huntingtin-interacting protein 1 coiled-coil domain. *Acta Crystallogr D Biol Crystallogr* **66**: 314–318.
- Wilbur JD, Hwang PK, Ybe JA, Lane M, Sellers BD, Jacobson MP, Fletterick RJ, Brodsky FM. 2010b. Conformation switching of clathrin light chain regulates clathrin lattice assembly. *Dev Cell* **18**: 841–848.
- Wilde A, Beattie EC, Lem L, Riethof DA, Liu SH, Mobley WC, Soriano P, Brodsky FM. 1999. EGF receptor signaling stimulates SRC kinase phosphorylation of clathrin, influencing clathrin redistribution and EGF uptake. *Cell* **96**: 677–687.
- Wilson GM, Fielding AB, Simon GC, Yu X, Andrews PD, Hames RS, Frey AM, Peden AA, Gould GW, Prekeris R. 2005. The FIP3-Rab11 protein complex regulates recycling endosome targeting to the cleavage furrow during late cytokinesis. *Mol Biol Cell* **16**: 849–860.
- Wirtz-Peitz F, Nishimura T, Knoblich JA. 2008. Linking cell cycle to asymmetric division: Aurora-A phosphorylates the Par complex to regulate Numb localization. *Cell* **135**: 161–173.
- Ybe JA, Ruppel N, Mishra S, VanHaaften E. 2003. Contribution of cysteines to clathrin trimerization domain stability and mapping of light chain binding. *Traffic* **4**: 850–856.
- Ybe JA, Mishra S, Helms S, Nix J. 2007a. Crystal structure at 2.8 Å of the DLLRKN-containing coiled-coil domain of huntingtin-interacting protein 1 (HIP1) reveals a surface suitable for clathrin light chain binding. *J Mol Biol* **367**: 8–15.
- Ybe JA, Perez-Miller S, Niu Q, Coates DA, Drazer MW, Clegg ME. 2007b. Light chain C-terminal region reinforces the stability of clathrin heavy chain trimers. *Traffic* **8**: 1101–1110.
- Ybe JA, Clegg ME, Illingworth M, Gonzalez C, Niu Q. 2009. Two distantly spaced basic patches in the flexible domain of huntingtin-interacting protein 1 (HIP1) are essential for the binding of clathrin light chain. *Res Lett Biochem* **2009**: 256124.
- Ybe JA, Fontaine SN, Stone T, Nix J, Lin X, Mishra S. 2013. Nuclear localization of clathrin involves a labile helix outside the trimerization domain. *FEBS Lett* **587**: 142–149.
- Zhang F, Zang T, Wilson SJ, Johnson MC, Bieniasz PD. 2011. Clathrin facilitates the morphogenesis of retrovirus particles. *PLoS Pathog* **7**: e1002119.

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